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A balancing act for chemical purity

Anyone who has ever tried to use the spray from a hose to keep a Ping-Pong ball suspended in the air knows that it's hard to keep the ball in place for very long. This kind of stability problem also faced biochemist Patrick H. O'Farrell when he first thought of his scheme for purifying proteins by balancing the flow of a liquid against the opposing pull of an electrical force. Nevertheless, he found an answer, and the result is a versatile purification method that may be of considerable interest to biotechnology companies and others interested in recovering, on a large scale, pure compounds from complex mixtures.

O'Farrell, a researcher at the University of California at San Francisco, describes the results of his "initial investigations" into this new group of separation methods in the March 29 SCIENCE.

Essentially, O'Farrell combines two well-known and widely used separation methods: electrophoresis and chromatography. In electrophoresis, large molecules with a net electric charge migrate through a solution under the influence of an applied voltage. Different proteins (the solute), for instance, move at different speeds. In chromatography, a solution trickles through a bed of tiny beads that selectively retard the passage of molecules. Again, different molecules move through at different speeds but rarely at rates that match those in electrophoresis. By combining these two separation techniques, O'Farrell invented a purification method that he calls "counteracting chromatographic electrophoresis."

The trick to making this technique work is careful selection of the porous resin beads or gel beds (the chromatographic matrix) that go into a separation column. "A chromatographic matrix can influence solute movement with a flowing solvent differently from the way it influences solute electrophoresis," reports O'Farrell, "and thereby can bring about a balance between these opposing forces."

In its simplest form, the technique involves packing a glass cylinder with an upper layer of beads through which a particular protein passes quickly and a lower layer through which it travels more slowly. The applied voltage is carefully selected so that it drives molecules upward at a rate greater than the protein's flow rate downward through the lower bed, but less than its flow rate in the top layer. Hence, the protein is concentrated at an equilibrium position at the interface between the two different gel beds.

"The approach is very general and adaptable," says O'Farrell. By adjusting the voltage or by selecting different types of beads, different proteins can be concentrated. It is also possible to create a column with several interfaces so that more than one compound can be purified at the same time.

Although O'Farrell's paper is only now appearing in a scientific journal, he actually invented and patented this separation technique several years ago. "It's just now that the people involved in biotechnology are beginning to realize the importance of purification procedures for eventually producing something for market," he says. "So, all of a sudden, there's this big interest in this area."

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By Ivars Peterson

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